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Abstract
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Map of Plasmid pRAL1

We reported previously the development of a general method for cloning Neurospora nuclear genes by sib selection, using a library of N. crassa genomic DNA fragments in plasmid pRAL1 (Akins and Lambowitz Mol. Cell. Biol. 5:2272-2278, 1985). Fig. 1 is a revised map of plasmid pRAL1.

The size of the plasmid is measured more accurately as 4.7 kb, rather than 4.4 kb reported previously. In addition, the position of the EcoR1 site in the qa-2" gene was indicated incorrectly in the previous map. The sizes of the EcoR1 fragments are 2.8 and 1.85 kb.

We and others have now cloned at least ten genes using the pRAL1 library: nic-1 and inl (Akins and Lambowitz, Molec. Cell. Biol. 5:2272-2278, 1985), cyt-18 (Akins and Lambowitz, unpubl.), cyt-(297-24) Kuiper, de Vries, Akins and Lambowitz, unpubl.), cyt-4 (Serizawa, Akins and Lambowitz, unpubl.), cyt-(289-4) (Kubelik and Lambowitz, unpubl.), his-2 (Akins, Lambowitz and Kinsey, unpubl.), van (Mann, Metzenberg, Akins and Lambowitz, unpubl.), cys-3 (Paletta, Marzluf, Akins and Lambowitz, unpubl.) and met-7 (Dr. M. Case, University of Georgia, personal communication). The library is available to all investigators. This work supported by NIH grant GM23961.

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Chambers, J.A.A. and V.E.A. Russo

Isolating RNA is easy and fun

We have found that the common phenol or guanidium thiocyanate based procedures for isolating RNA simply do not repay the effort for the yields involved (0.2-0.3 mg total RNA per g fresh weight with guanidium salts). A number of observations led us to examine and slightly modify a procedure originally developed for tissue culture cells (Auffray and Rougeon, 1979, Eur. J. Biochem. 107:303). This somewhat unusual procedure is based upon lithium chloride/urea solubilization of the cellular contents and the ultimate precipitation of RNA at high ionic strength. The combination of effective solubilization, inhibition of RNase activity, effective precipitation of RNA, and a minimum of handling results in higher yields (0.5-1 mg RNA per g fresh wt.) for much less effort.

Precautions

General precautions for handling RNA are applied. With the exception of the lithium chloride/urea solution - which is prepared fresh - all aqueous solutions are autoclaved before use. We are not enthusiastic about the use of diethyl pyrocarbonate (DEP) as a cure-all for RNAse problems and in any case it is not compatible with Tris buffers. The lithium chloride/urea solution can be neither autoclaved nor treated with DEP. The combination of chaotropism and high ionic strength seems to be completely effective in inhibiting RNases.

Solutions

6M urea, 3M LiCl - freshly prepared, approx. 20 ml per g wet wt.
0.5% SDS, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA
10 M Ammonium acetate
Chloroform/isoamyl alcohol (24:1 v/v), Absolute ethanol
Liquid nitrogen

Method

1. Mycelium is harvested, washed, pressed dry and frozen in liquid nitrogen. Our typical growth conditions are an inoculum of 2x10^5 conidia ml^-1 in Vogel's medium with 2% sucrose as carbon + 0.2% Tween 80 incubated at 34° C for 24 h in a shaking incubator at 200 rpm.